Direct and indirect effects of climatic changes on vegetation productivity and species composition of permafrost peatlands

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2012

document version
Publisher's PDF, also known as Version of record

Chapter IV

A Frozen Feast:
Thawing permafrost increases plant-available nitrogen in subarctic peatlands

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Global Change Biology 18 (2012) 1998-2007

Abstract

Many of the world's northern peatlands are underlain by rapidly thawing permafrost. Because plant production in these peatlands is often nitrogen (N)-limited, a release of N stored in permafrost may stimulate net primary production or change species composition if it is plant-available. In this study, we aimed to quantify plant-available N in thawing permafrost soils of subarctic peatlands. We compared plant-available N-pools and -fluxes in near-surface permafrost (0-10 cm below the thawfront) to those taken from a current rooting zone layer (5-15 cm depth) across five representative peatlands in subarctic Sweden. A range of complementary methods was used: extractions of inorganic and organic N, inorganic and organic N-release measurements at 0.5 and 11 °C (over 120 days, relevant to different thaw-development scenarios) and a bioassay with *Poa alpina* test plants. All extraction methods, across all peatlands, consistently showed up to seven times more plant-available N in near-surface permafrost soil compared to the current rooting zone layer. These results were supported by the bioassay experiment, with an eight-fold larger plant N-uptake from permafrost soil than from other N-sources such as current rooting zone soil or fresh litter substrates. Moreover, net mineralisation rates were much higher in permafrost soils compared to soils from the current rooting zone layer (273 mg N m^{-2} and 1348 mg N m^{-2} per growing season for near-surface permafrost at 0.5 °C and 11 °C respectively, compared to -30 mg N m^{-2} for current rooting zone soil at 11 °C). Hence, our results demonstrate that near-surface permafrost soil of subarctic peatlands can release a biologically relevant amount of plant available nitrogen, both directly upon thawing as well as over the course of a growing season through continued microbial mineralisation of organically bound N. Given the nitrogen-limited nature of northern peatlands, this release may have impacts on both plant productivity and species composition.

Introduction

Many of the world's northern peatlands, which contain one-third of the global soil organic carbon pool (Gorham 1991), are underlain by permafrost (Tarnocai *et al.* 2009). Recently, it was shown that thawing of permafrost soils occurs over large geographical areas due to climatic warming (ACIA 2004, IPCC 2007). Due to large stocks of carbon and nutrients in these frozen soils this has a potential to feed back to global biogeochemical cycles (Tarnocai *et al.* 2009, Kuhry *et al.* 2010).

Low soil nutrient availability limits plant growth at high-latitudes and in peatlands in particular (Aerts *et al.* 1992, Berendse & Jonasson 1992, Chapin *et al.* 1995, Hobbie *et al.* 2002, Van Wijk *et al.* 2004). Although peatland soils contain large stocks of nitrogen (Limpens *et al.* 2006), these stocks are largely tied up in unavailable, organic N forms (Rydin & Jeglum 2006), immobilized in microbial biomass (Jonasson *et al.* 1996) or in permafrost soils, and are therefore mostly not available to plants.

Thawing of permafrost soils, however, might cause a release of plant-available N into N-limited high-latitude peatlands. Although little is known about plant-available N concentrations in permafrost soils, increased uptake of nitrogen by plants in Alaskan thermokarst areas (Schuur *et al.* 2007), and increased organic nitrogen concentrations in Arctic rivers as a result of permafrost

degradation (Frey & McClelland 2009) suggest that plant-available nitrogen release from thawing permafrost is occurring in some areas. Also, N measurements in Yedoma soils of Northeast Siberia suggest a biologically relevant N release upon thaw (Mack *et al.* 2010). Despite this circumstantial evidence, little is known about the actual quantity and particularly about the quality of N stored in permafrost soils underlying peatlands.

Two historic processes may account for the high amount of potentially plant-available N in permafrost soil and cause two different mechanisms and time-spans through which plant-available N may be released from thawing near-surface permafrost soil. Firstly, the amount of dissolved, but frozen, plant-available N in near-surface permafrost (just below the thaw-front) may be high due to leaching of dissolved N from the active layer into the perennially frozen ground at the end of summer over the course of decennia (Mackay 1983, Allard & Rousseau 1999, Kokelj & Burn 2003) and because of a spatio-temporal separation of microbial N mineralisation and plant N-uptake (cf. Hobbie & Chapin 1996). This N will be readily available to plants immediately upon thaw and will most likely be used up rapidly by plants and/or microorganisms. A second release of plant-available N may come from organically bound yet potentially mineralisable N, accumulated as a result of historical conditions unfavourable to decomposition (cf. Weintraub & Schimel 2003). Mineralisation of this N may be activated in defrosted soils, creating a longer-term, sustained source of plant-available N.

The aim of this paper is therefore to provide insight both into the amount of readily plant-available N in thawing permafrost of peatlands in subarctic Sweden and into the longer-term release of plant-available N from permafrost soil through N-mineralisation. We hypothesized that near-surface permafrost soil of subarctic peatlands can release a biologically relevant amount of (1) readily plant-available N directly upon thawing (i.e. detectable with traditional extraction methods and as measurable plant-uptake) and of (2) plant-available N through mineralisation after thawing over the course of a growing season. Because this is the first study providing data on plant-available N release from subarctic peatland permafrost soils, we sampled a range of representative peatlands to estimate spatial variability. Given that methods for measuring plant-available N are debated (e.g. Van Duren & Pegtel 2000), we used two complementary methods to obtain a robust measure of directly biologically available N and test our hypothesis (1): extractions of inorganic and organic N and a bioassay with *Poa alpina* test-plants to measure net plant N-uptake (Quested *et al.* 2003, Dorrepaal *et al.* 2007). To assess the relative significance of this N-release from thawing permafrost soil, we compared it with the release from two other important N sources: current rooting zone soil and leaf litter.

To test our hypothesis (2), we performed a mineralisation incubation experiment at two temperatures. Because thawing of permafrost peatlands can progress as a mere gradual thickening of the active layer (ACIA 2004) or as thermokarst development and subsequent peatland collapse (Beilman & Robinson 2003, ACIA 2004, Luoto *et al.* 2004), the temperature aspect of two thaw-development scenarios was mimicked in order to compare its potential effects on the mineralisation of N from thawing of permafrost soil. Although multiple factors limiting N-mineralisation such as hydrology, biochemistry, oxygen availability and soil temperature (Robinson 2002, ACIA 2004, Schuur *et al.* 2008) may be affected differentially by such diverging thaw scenarios, we limit our research to the impact of temperature, an important determinant of microbial nitrogen mineralisation rates (Robinson 2002, Jones *et al.* 2009, Wallenstein *et al.* 2009). The incubation temperatures were 0.5 °C (deepening active layer scenario) and 11 °C (degrading palsa scenario) for near-surface permafrost and 11 °C for current rooting zone soil,

based on measurements of temperatures just above the thaw front during core extraction (0.5 °C) and on average ambient field summer rooting-zone soil temperatures (11 °C). Furthermore, because microbial immobilization of inorganic N can be a strong N-sink in organic soils, we also measured microbial biomass N.

Methods

Sampling area

To characterize spatial variability in N availability, soil samples were taken from five ombrotrophic permafrost peatlands (Table 1) in the Torneträsk region in the northernmost part of Sweden, which lies within the zone of sporadic permafrost (Brown *et al.* 1998). Mean annual precipitation in this area was around 350 mm in the most recent decade and mean annual air temperature around -0.6 °C (meteorological data 1999-2008, Abisko Scientific Research Station), but there is evidence that since 2000 the long-term trend of mean annual temperature has significantly exceeded the 0 °C threshold (Callaghan *et al.* 2010). The temperature of the permafrost in the study area is only a few degrees below zero (Akerman & Johansson 2008, Johansson *et al.* 2011), and mean summer soil temperature in the rooting zone (at 5-15 cm) of these peatlands is around 11 °C (unpublished data Dept. of Systems Ecology, VU University Amsterdam). In all five peatlands the vascular vegetation was low and open and dominated by the peatmoss *Sphagnum fuscum* (Schimp.) H. Klingg. The exact sampling locations were chosen for the presence of a living *S. fuscum* carpet and the vascular plant species *Empetrum hermaphroditum* Hagerup, *Andromeda polifolia* (evergreen dwarfshrubs), *Rubus chamaemorus* (forb), *Betula nana* and *Vaccinium uliginosum* (deciduous dwarf shrubs).

Soil sampling

Soil samples were taken in the second week of September 2008, within one week of the time of maximum seasonal thaw depth. A rectangular (5x6 cm) hardened stainless steel hand corer with a length of 1 m was driven through the active layer and forced into the frozen soil. Three to seven cores were extracted for each of the five peatlands (Table 1). Near-surface permafrost samples were cut 0-10 cm below the thawfront. For comparison, an equal volume of current rooting zone soil was taken from each soil core at 5-15 cm below the soil surface, which is where the bulk of the plant roots are concentrated (Rydin and Jeglum, 2006). Therefore, this is the most appropriate part of the active layer for plant N-uptake with which to compare the N availability for plants from near-permafrost. Therefore, this is the most relevant part of the active layer for plant N-uptake and thus to compare the N availability for plants from near-permafrost with.

Table 1. The peatland names, mean active layer thickness (ALT) and number of cores taken per peatland. The depth of the peat is similar (about 90 cm) at three of the five peatlands (Storflaket, Kursflaket and Abisko Naturreservat) (Akerman & Johansson 2008) and is at least > 70 cm at Stordalen and Torneträsk.

Peatland name	*Coordinates*		*Mean ALT*	*n° of cores taken*
A. Stordalen	68°21.428'N	19°03.181'E	50 cm	7
B. Torneträsk	68°13.423'N	19°44.621'E	56 cm	4
C. Storflaket	68°20.836'N	18°58.398'E	54 cm	5
D. Abisko	68°21.533'N	18°48.594'E	50 cm	5
E. Kursflaket	68°21.011'N	18°52.324'E	52 cm	3

The position of the thawfront was determined separately at three replicate points around each sampling location by probing a 0.5 cm diameter graduated stainless steel rod into the soil to refusal, because active layer thickness (ALT) as determined by probing was generally larger than when visually estimated from the soil cores. Mean ALT per peatland is presented in Table 1. The soil samples were transported unthawed to the laboratory and stored at -18 °C. Subsamples of each sample were analysed for moisture content, bulk density (dry mass per volume; Table SI 1, Supporting Information) and elemental N (by dry combustion of ground samples with a Flash EA1112 elemental analyser, Thermo Scientific, Rodana, Italy) for which the subsamples were oven dried at 60 °C for 48 hrs. Prior to all analyses, roots (>0.5 mm) were removed from the replicate soil samples and each replicate was homogenized. This was done in a cool room (11 °C) in order to minimize moisture loss and microbial mineralisation.

Nitrogen pool measurements

Nitrogen availability to plants is generally measured and expressed as extractable inorganic nitrogen (NO_3- and NH_4+). However, low molecular mass forms of organic nitrogen (e.g. amino acids) can also be considered plant-available, especially in (sub)arctic systems where ectomycorrhizal organic N uptake is abundant (McKane *et al.* 2002, Nasholm *et al.* 2009). Therefore, a range of complementary measures was used to compare the release of plant-available N from near-surface permafrost soils of subarctic peatlands to that from current rooting zone soil.

Nitrogen extractions

Both plant-available extractable inorganic as well as organic nitrogen content were determined by means of chemical extractions. Extractable inorganic N was determined after shaking 5 gram fresh soil for two hours in 25.0 ml 1 M KCl. Extractable organic N was calculated by subtraction of inorganic N from total extractable N. For these calculations inorganic N was also measured in a 0.5 M K_2SO_4-extract (5 gram fresh soil per 25 ml), and total extractable N of the same sample was determined after potassium persulfate digestion (Cabrera & Beare 1993). Ammonium and nitrate (inorganic N) concentrations in all extracts were measured using a SA-40 auto-analyser (Skalar, Breda, The Netherlands). Values were multiplied with their bulk density (Table SI 1) and expressed as mg N per m^2 for the layer investigated (10 cm soil depth).

Bioassay

In addition to the extractions and in order to assess the plant availability (i.e. uptake) of N in the presence of other nutrients as well as phytotoxic substances (e.g. polyphenols), we measured plant-available N by means of a bioassay (Clements & Goldsmith 1924).

We used the perennial grass *Poa alpina* (Mossberg *et al.* 1992) as a test species (henceforth 'phytometer') (Quested *et al.* 2003, Dorrepaal *et al.* 2007). The phytometers were picked directly from viviparous *Poa alpina* parent plants, which were collected in Abisko, northern Sweden (68°21'N, 18°49'E) in August 2008. A positive control experiment with increasing levels of N-addition confirmed the strong nutrient-limitation of *Poa alpina* phytometer growth (Fig. SI 1, Supporting Information). Upon planting, all phytometers received approximately 10 ml of a liquid mixture of peat and fine roots collected at the *Poa alpina*-parent community to provide an inoculum of natural soil organisms.

The phytometers (one per pot, n =13 per treatment) were grown for a period of 74 days and plant N-uptake from permafrost soil, current rooting zone soil, and a representative mix of fresh peatland litter were compared. The latter two treatments were added to test whether permafrost soil is a biologically relevant source of nitrogen. Therefore, phytometers were grown on a mixture of sand with either fresh, thawed soil (either permafrost or current rooting zone) or with a leaf litter substrate, equivalent to 3 g dry weight of each substrate per pot (0.6 dm^3). The litter treatment consisted of a mixture of the two common subarctic peatland species *Rubus chamaemorus* and *Betula nana* (resp. 2.4 g and 0.6 g). An additional control treatment consisted of sand only. The soil and litter samples for the bioassay were collected from one representative peatland (Peatland A, Fig. 1; Table SI 1, Supporting Information) in September and processed as described above.

The bioassay experiment was performed in a greenhouse at 14 °C and a random block design of pot positions was applied and updated bi-weekly. The phytometers were watered two times per week with de-ionized water. Upon harvest, the plants and roots (Fig. SI 2, Supporting Information) were cleaned, oven dried (60 °C, 48 h) and weighed. The total amount of elemental N per phytometer was determined in ground material by dry combustion with a Flash EA1112 elemental analyser (Thermo Scientific, Rodana, Italy), after which plant N-uptake was calculated by multiplying the plant N concentration with the total dry weight of the phytometer and expressed as N-uptake per gram substrate (permafrost or current rooting zone soil or litter) (μgN/gDW).

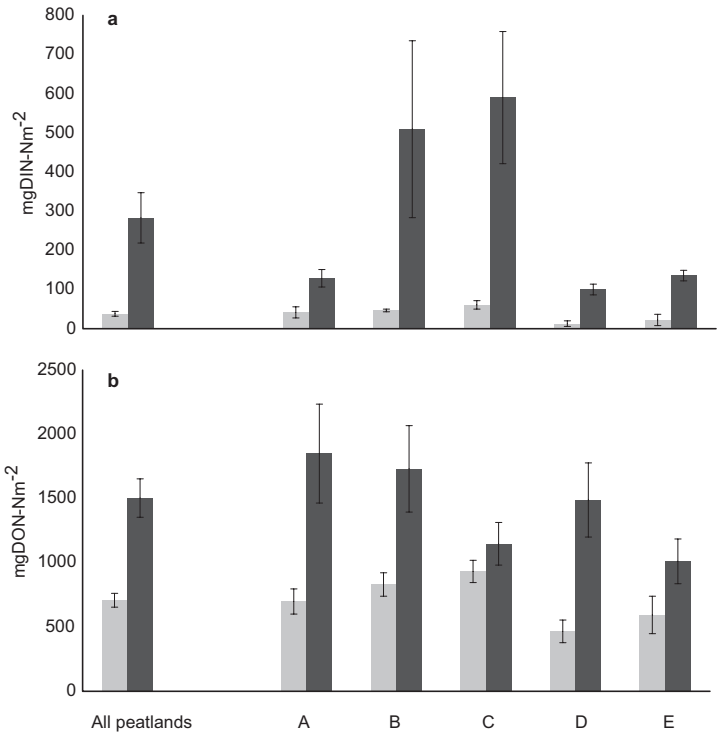

Figure 1. Extractable inorganic N (a) and extractable organic N (b) in current rooting zone soil (grey bars) and in near-surface permafrost (black bars) soil of five subarctic peatlands (Table 1) at the end of the growing season. Data are means ± SE, expressed per cm soil depth, with 3-7 replicates per peatland.

Mineralisation incubations: simulation of two permafrost thaw scenarios

To assess the longer-term plant-available N release from near-surface permafrost soil, two temperature scenarios relevant to different thaw-development in permafrost soils were mimicked by mineralisation incubations. In the first scenario we recreated a situation in which the defrosted near-surface permafrost soil temperature increases only to little above 0 ºC due to the continuing insulation provided by an overlying intact peat layer: the 'Deepening active layer' scenario (I). The second scenario sought to simulate the temperature in a situation where the peatland is subject to severe degradation and collapse and formerly frozen soil is exposed to current rooting zone soil temperatures: the 'Degrading palsa' scenario (II). Scenario I incubation temperatures were 0.5 °C for near-surface permafrost and 11 °C for current rooting zone soil, based on average ambient field summer rooting-zone soil temperatures and measurements of temperature at the thaw front during core extraction (equalling 0.35 °C +/- 0.2 °C). Under Scenario II, soil from both depths was incubated at average ambient field summer rooting-zone soil temperature (11 °C). The samples were incubated for 120 days for both scenarios, similar to the duration of the growing season in this area (Karlsson & Callaghan 1996).

At both temperatures, 15 gram fresh soil per sample was incubated in 50 ml pots and the positions of the bottles within the dark incubation chambers were randomized. Each pot was covered by parafilm with three small openings to minimize moisture loss but allow for gas exchange. Moisture loss was measured weekly by weighing the pots and when necessary returned to the initial weight with de-ionised water. Measurement of inorganic and organic N was performed as described above, at the beginning and the end of the experiment. In addition, the microbial biomass N pool was determined, for which 5 g subsample was chloroform-fumigated for 24 hours at ~20 °C in a darkened desiccation jar, and then extracted after two hours shaking in 25.0 ml 0.5 M K_2SO_4. Similarly, a second non-fumigated sample was extracted for determination of total extractable non-microbial N. Both samples were then oxidized by potassium persulfate digestion for determination of total extractable N (Cabrera & Beare 1993). Microbial biomass pool sizes were calculated as the differences between fumigated and non-fumigated extracts, using correction factor KE*N* = 0.40, representative for organic soils, to account for microbial tissue N that is not released by exposure to chloroform (Jonasson *et al.* 1996).

Calculation of mineralisation, immobilization and microbial biomass N dynamics

Net mineralisation and immobilization as well as net change in extractable organic N and microbial biomass N was calculated as the differences between the values obtained at the start and the end of the experiment.

Statistical analysis

All data were tested for normality and homogeneity of residual variances by visual inspection of residual and probability plots. Log-transformation improved the homogeneity of residual variances for $N\text{-}NO_3$, $N\text{-}NH_4$, inorganic N (NO_3 + NH_4), organic N and total N values and of the bioassay data. For all other variables residual variance was approximately normal and homogeneous.

The effects of the sampling depth (current rooting zone or near-surface permafrost) and sampling site (peatland) on all soil variables were analysed using repeated measures (RM-) ANOVAs, with

'depth' within a core as the within-subject factor and 'peatland' as the between-subject factor. The effects of the five different peatlands were separated by a Tukey's HSD post-hoc test. In a similar RM-design with 'peatland' as the between-subject factor, mineralisation rates were analysed with rates of current rooting zone soil at 11 °C vs. rates of near-surface permafrost soil at 0.5 °C as within-subject factor for Scenario I. For Scenario II, rates of current rooting zone soil at 11 °C vs. rates of near-surface permafrost soil incubated at 11 °C were applied as within-subject factor. The bioassay data were analysed with a one-way ANOVA between the four levels of substrate treatment and Tukey's HSD post-hoc test to identify differences in treatment means. All analyses were performed with SPSS 15.0 for Windows.

Results

Higher values of plant-available nitrogen were found in near-surface permafrost soils compared to soil taken from the current rooting zone (5-15 cm) independent of the measurement method used.

The amount of readily plant-available inorganic N was seven times larger in permafrost soil than in an equal volume of current rooting zone soil (Fig 1a). The inorganic N fraction constituted around 0.09% of the total N pool for both current rooting zone soil and the permafrost soil samples, due to a larger total N pool in near-surface permafrost soil (Table 2). In both soil layers only a small fraction of inorganic N was NO_3-N; the main part was NH_4-N (79% in current rooting zone soil and 89% in permafrost soil) (Table 2). The amount of readily plant-available organic N was about two times larger in the permafrost soil than in the current rooting zone soil layer (Fig. 1b). Permafrost soil therefore had a three times higher ratio of plant-available inorganic to organic N than current rooting zone soil. There were significant differences in the amount of plant-available inorganic N across the five different peatlands, but the overall pattern of higher N values for permafrost soils was consistent and highly significant across all peatlands (Table 2).

Measurements of net phytometer N-uptake from permafrost soil, current rooting zone soil and fresh leaf litter yielded a strikingly similar pattern in plant-available N. Mean plant N content was respectively 189, 113, 147 and 1072 μgN for plants grown on the control, the current rooting zone soil, the peatland litter mix and the near-surface permafrost treatments. When expressed as mean uptake per gram dry weight of added substrate, we found over eight-fold larger net N-uptake by phytometers grown on sand mixed with near-surface permafrost soil than by phytometers grown on sand mixed with either current rooting zone soil or fresh leaf litter ($P < 0.001$)(Fig. 2).

In response to Scenario I ('Deepening active layer'), significantly larger net mineralisation rates were observed in permafrost samples than in rooting zone samples (Fig. 3a). Also, net consumption rates of extractable organic N were six times higher in the current rooting zone samples than in permafrost samples (Fig 3b). Surprisingly, although initial microbial biomass values were higher in current rooting zone samples than in permafrost samples (Fig. 4), there was no significant effect of depth on changes in microbial N under Scenario I. This indicates similar net microbial N immobilisation rates (around 0.8 g N m^{-2} per growing season) at dissimilar incubation temperatures in near-surface permafrost and current rooting zone soil (incubated at 0.5 °C and 11 °C respectively, Fig. 4).

Table 2. Averages, standard errors (SE) and F- statistics for repeated-measures ANOVAs for the effects of depth (current rooting zone, permafrost) and sampling location (peatland) on the availability of different nitrogen species. Values are expressed per 10 cm soil depth. The sampling location (five peatlands) was taken as between-subject factor and depth (current rooting zone (AL), permafrost (PF)) as within-subject factor. All N values were log-transformed before analysis, and $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

	Mean		SE		F-values		
Variable	**AL**	**PF**	**AL**	**PF**	**Depth**	**Peatland**	**Depth X Peatland**
Initial N pools ($mgNm^{-2}$)							
$N\text{-}NO_3$	8	31	2	6	15.37***	3.61*	1.39
$N\text{-}NH_4$	30	252	5	66	55.86***	5.69**	2.73
DIN ($N\text{-}NO_3$ + $N\text{-}NH_4$)	38	283	6	64	55.41***	6.13**	0.54
DON	708	1504	54	150	21.94***	1.97	1.27
Nmic	2318	974	235	229	14.12***	2.13	1.82
Mineralisation/immobilization/change rates ($mgNm^{-2}$ per growing season)							
Scenario I: AL 11°C vs. PF0.5°C							
DIN	-30	273	6	122	6.20*	1.33	1.39
DON	-426	-69	39	115	10.11**	1.37	1.05
Nmic	835	802	116	235	0.00	1.61	1.45
Scenario II: AL 11°C vs. PF 11°C							
DIN	-30	1348	6	351	8.91**	3.78*	4.23*
DON	-426	90	39	111	19.29***	0.77	1.62
Nmic	835	1492	116	284	4.78*	1.05	0.54

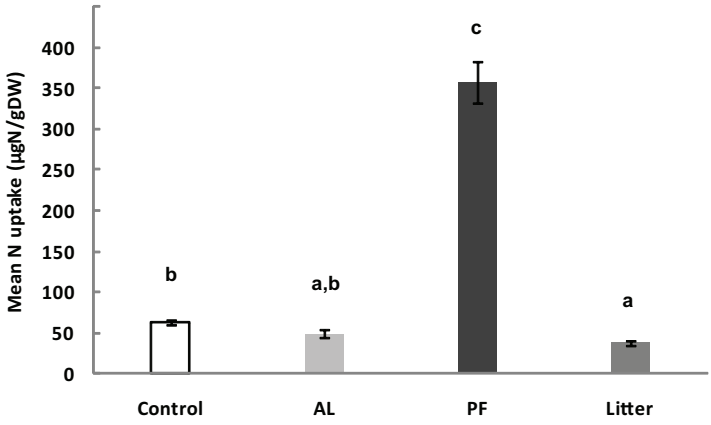

Figure 2. Phytometer plant-available N expressed as N-uptake per gram substrate (DW): near-surface permafrost soil (PF), current rooting zone soil (AL) or litter compared with control plant N-uptake without added substrate. Data are means ± SE, $n = 13$ per treatment, $P < 0.05$.

Under Scenario II, where samples from both soil layers had been exposed to ambient rooting zone temperatures ('Degrading palsa'), we found even larger differences in changes in N availability between the near-surface permafrost and current rooting zone samples. Firstly, a five-fold larger net release of extractable inorganic N was observed from permafrost samples

incubated at 11 °C (Scenario II) compared to permafrost samples incubated at 0.5 °C (under Scenario I), whereas only a net immobilization of inorganic N was observed in the current rooting zone soil samples incubated at 11 °C (Fig. 3a). In contrast, in the current rooting zone, similar rates of net immobilization occurred at 0.5 °C (results not shown). Secondly, extractable organic N change in the permafrost samples was 90 mgN m^{-2} as opposed to -426 mgN m^{-2} in an equal volume of current rooting zone soil per simulated growing season under Scenario II (Fig. 3b). In contrast to Scenario I, the net immobilisation rate of plant available N into microbial biomass was significantly larger in permafrost samples incubated at 11 °C compared to that in the current rooting zone soil samples incubated at 11 °C (Scenario II) (Fig. 4, Table 2). This indicates that total nitrogen fluxes in the permafrost samples at scenario II had even been higher. The overall significant effect of sampling depth on inorganic and organic pool change rates was consistent across all peatlands under both Scenario I and II (Table 2).

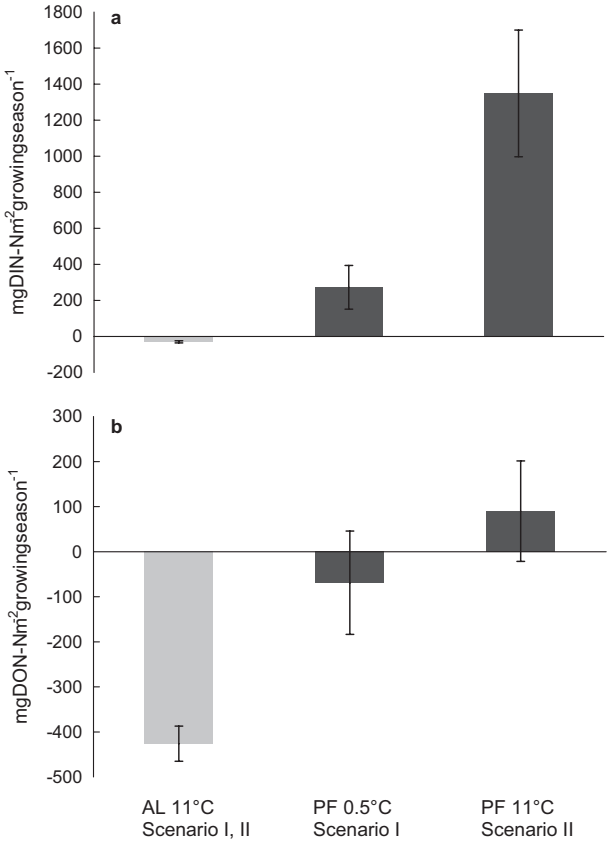

Figure 3. Net rate of change in extractable inorganic and organic N in current rooting zone soil (grey bars) vs. near-surface permafrost soil (black bars) under both a 'deepening active layer' scenario (I) and a 'degrading palsa' scenario (II) over one growing season. Data are means of five peatlands ± SE.

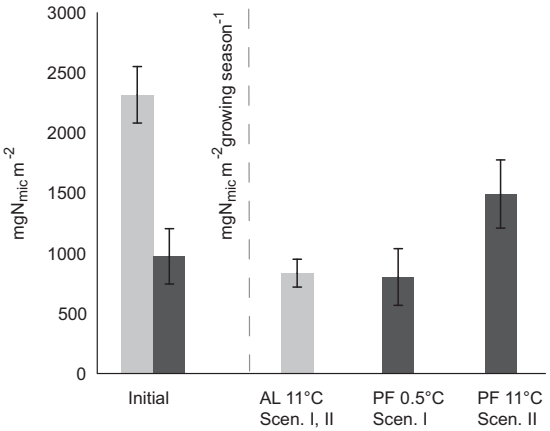

Figure 4. Pool sizes at the start of the incubation (initial) and rate of change in microbial biomass N (N_{mic}) in current rooting zone soil (grey bars) vs. near-surface permafrost soil (black bars) under both a 'deepening active layer' scenario (I) and a 'degrading palsa' scenario (II) over one growing season. Data are means of five peatlands ± SE.

Discussion

This study shows consistently higher concentrations of plant-available N in near-surface permafrost soils than in current rooting zone soils of subarctic peatlands. Moreover, we show that plant-available N released from near-surface permafrost can originate both from a limited but readily plant-available N pool (historically accumulated in frozen layers) as well as from N mineralisation of freshly thawed soil over the course of a growing season.

To our knowledge, this is the first study providing data on plant-available N in near-surface permafrost of subarctic peatlands. We do realize that the data may not be extrapolated directly to the field situation. Instead, our data serve as a first estimate on the relevance of plant-available N from permafrost soils. Below we will discuss our findings and their potential implications for the nutrient dynamics of sub-arctic peatlands underlain by thawing permafrost.

Fast-food: readily available plant-available N pools in near-surface permafrost soil

We found plant-available N concentrations in near-surface permafrost soils that were up to seven times higher than in current rooting zone samples (Fig. 1a, b). However, the samples were taken at the time of maximum thaw, i.e. at the end of the growing season. Higher plant-available N pool sizes might be expected in the rooting zone of tundra soils earlier in the season, i.e. directly after spring thaw but before plant uptake (Hobbie & Chapin 1996, Larsen *et al.* 2007). This would imply that the difference between the two soil layers may be smaller earlier in the growing season. Indeed, comparative measurements in the current rooting zone in mid-June and mid-September showed three-fold higher early season inorganic N values in the current rooting zone, while organic N values remained constant (results not shown). However, even taking these early-season N values into consideration, the plant-available N pool sizes are still up to 100% larger in near-surface permafrost soil than in soil taken from the current rooting zone layer (5-15 cm).

In line with the extraction results, plant N-uptake as measured by means of the bioassay was significantly stimulated by the near-surface permafrost soil substrate. In contrast, current rooting zone soil and fresh litter had very little or, in the case of plant litter, even negative effects on phytometer growth and N-uptake (Fig. 2), although these are considered the two primary plant-available N-sources in subarctic mires (Rosswall & Granhall 1980). Apparently, these N-sources contain less readily plant-available N, may have a slower turnover due to more structural carbon-substrates (i.e. the litter samples), or may have had phytotoxic (related to a high release of phenolic compounds) or immobilising effects that negatively affected plant N-uptake (Robinson 2002, Dorrepaal *et al.* 2007). Such negative effects did not occur for the phytometers which were grown on the near-surface permafrost substrate. However, it should be realized that in the longer term net litter N mineralisation will occur (Cadish & Giller 1997). Nevertheless, this comparison of net phytometer N-uptake from near-surface permafrost soil with two important other nutrient sources in subarctic peatland ecosystems corroborates our findings from the chemical extractions that thawed near-surface permafrost soil might provide an important 'new' plant-available N-source.

Our data thus show a consistent pattern of a significantly larger amount of readily plant-available N in near-surface permafrost than in current rooting zone soil, independent of measurement method or peatland. This N will become available immediately upon thawing, but will most likely be used up shortly after thawing.

Slow-food: longer-term release of plant-available N from permafrost soil

The relatively high net N-mineralisation rates in near-surface permafrost soil (Fig. 3a) over the course of a simulated growing season show that plant-available N in near-surface permafrost soil not only consists of historically accumulated, dissolved readily plant-available N, but also contains a considerable amount of organically-bound N. Upon thawing, this organically-bound N can be mineralised at significantly higher rates in near-surface permafrost soil than in current rooting zone soil, even under our most conservative temperature scenario (Scenario I).

Furthermore, the difference between mineralisation rates under Scenario I and II showed that the mineralisation of N in near-surface permafrost is highly temperature sensitive, with a five times increase over 0.5 ºC to 11 ºC, which is high, but not unrealistic (Davidson & Janssens 2006). Even though care should be taken when comparing in vitro with in situ conditions, and noting that the two scenarios presented here included temperature effects only, the different responses to the two imposed scenarios demonstrate that the mode of thaw-development is an important determinant of permafrost-thaw effects on nutrient cycling.

The observed high mineralisation rates in near-surface permafrost soil point to the presence of a potentially active microbial community. Complementary enzyme analyses on the same samples corroborate this finding: all permafrost samples showed high potential aminopeptidase activities and three out of four analysed potential activities were significantly larger in permafrost samples than in current rooting zone samples (Table SI 2, Supporting Information). Additionally, a strikingly similar increase in microbial biomass N was observed in soil from both depths under Scenario I in spite of the 10 ºC lower incubation temperature of permafrost soil ('Deepening active layer', Fig. 4)). Under Scenario II ('Degrading palsa'), the absolute increase in microbial biomass N in permafrost soil was even almost twice as large as in current rooting zone soil despite the higher net N mineralisation rates (Fig. 4). The highly active microbial community in the near-

surface permafrost may partially explain the high temperature sensitivity of N mineralisation rates of these soils, since the temperature response is higher than would be expected based on enzyme responses to temperature alone (Weedon *et al.* 2012). It is well-established that microbial activity can occur at sub-zero temperatures (Panikov *et al.* 2006, Wallenstein *et al.* 2009), and our results are the first to suggest that a microbial community at the thawfront can indeed actively alter the nutrient status of subarctic peatlands.

The frozen feast released

Based on an observed one cm thaw of permafrost per year (Akerman & Johansson 2008) and the data presented in this study, we can provide a preliminary estimate of the amount of plant-available N release per year in the near-future from thawing permafrost in subarctic peatlands. Following a one-off release of 0.03 g N m^{-2}, a slow but continuous release of 0.03 g N m^{-2} yr^{-1} (dissolved inorganic) can be expected from thawing permafrost under Scenario I, and of 0.13 g N m^{-2} yr^{-1} under Scenario II (Table 2). Moreover, these values would be cumulative in the near-future because due to ever increasing active layer thickness, every year more soil is seasonally thawed and releasing plant-available N. Hence, with progressing permafrost-thaw, an increasing yearly contribution, from 0.03 - 0.13 g N m^{-2} yr^{-1} in the first year to 0.3 – 1.3 g N m^{-2} yr^{-1} in the tenth year could be expected (assuming constant decomposition and mineralisation rates, growing season length and soil temperatures). Even if the mineralisation rates of a thawed layer would decrease over time (which may be expected given the apparent labile nature of the organic compounds mineralised in the near-permafrost soil), these values are considerable compared with the 0.8 g N m^{-2} yr^{-1} net N mineralisation in northern peatland ecosystems (Rosswall & Granhall 1980). Net annual N mineralisation in northern peatland ecosystems could increase by up to 46% as observed in climate warming experiments (in the range of 0.3 - 6.0 ºC; Rustad *et al.* 2001). Furthermore, atmospheric deposition of N in northern Scandinavia is around 0.1-0.2 g N m^{-2} yr^{-1} (Dentener *et al.* 2006). This shows that although ambient net N mineralisation will most likely still be the main source of plant-available N under both Scenario I and under Scenario II, the potential plant-available N release through permafrost thawing is at least in the same order of magnitude as other climate-change related increases in N input in northern peatlands.

Whether nutrients released at the thawfront will really become available to plants will ultimately depend on the ability of the local vegetation to reach this N source. Obviously, this is most critical under Scenario I, where the peatland structure stays intact. Although little information about the activity of plant roots at the permafrost thaw front is currently available, the roots of some graminoids are known to grow at the surface of the frozen soil as this surface recedes down the soil profile towards the permafrost during summer (Bliss 1956, Callaghan *et al.* 1991). Also, roots of *Rubus chamaemorus* were observed at the permafrost thaw front during sampling for this study (pers. obs. F. Keuper). Hence, indeed some plant species may be capable of foraging the thawfront for nitrogen.

These observations suggest that a sustained release of nutrients at the thaw front might thus be expected to selectively benefit specific, deep-rooting species and thereby alter species composition. Although many fertilization experiments have been carried out in subarctic tundra indicating a likely change in the vegetation community (Parsons *et al.* 1995, Michelsen *et al.* 1996, Press *et al.* 1998, Robinson *et al.* 1998, Shaver *et al.* 2001, Haugwitz & Michelsen 2011, Lamb *et al.* 2011), the vegetation responses to a release of nutrients in deeper soil layers such as presented

here cannot be inferred in a straightforward manner from studies where fertilizer was applied at the soil surface.

In addition to the local implications of our results for the vegetation of northern peatlands, N release by thawing permafrost might have larger-scale impacts, as northern peatlands are an important carbon sink. Carbon release due to decomposition of plant litter and soil organic matter is exceeded by carbon assimilation in primary production in these ecosystems, but both processes are restricted by the availability of nitrogen (Chapin *et al.* 1995, Robinson 2002, Mack *et al.* 2004, Reich *et al.* 2006) due to low external nitrogen inputs. A permafrost-thawing induced increase in plant-available N might therefore stimulate biomass production (Rustad *et al.* 2001), and thus C-uptake. On the other hand, litter quality might increase (lower C:N ratio) (Aerts 1997), potentially stimulating decomposition (Bragazza *et al.* 2006). Over the longer term, an internal release of N from thawing permafrost is likely to speed up internal N and C cycling in northern peatlands, and potentially change their function as carbon sink, although the magnitude and direction is hard to predict without further understanding of plant and microbial responses to an increased deep N input.

Altogether, our results suggest that this 'new' climate-change induced N input into subarctic peatlands is indeed potentially available to plants and moreover similar in magnitude to other climate change induced increased N inputs such as increased mineralisation in surface soil layers (current rooting zone) and atmospheric deposition. These results from permafrost underlain peatlands are thus important when predicting the response of these nutrient-limited northern ecosystems to climate warming.

Acknowledgements

The authors would like to thank S. Marozava for her irreplaceable help during sampling and S. Sjögersten for her generous offer to (ab)use her peat corer, which made this research possible. Many thanks go to M. Johansson for helpful discussions and to the staff of the Abisko Naturvetenskapliga Station for technical assistance. Financial support was offered to FK by the Darwin Centre for Biogeosciences (grant 142.161.042) and ANS Scholarship 2008, and to RA by the Dutch Polar Program (ALW-NPP grant 851.30.023) and the EU-ATANS (grant FP6 506004).

Supporting Information

Table SI 1. Mean bulk density values (dry soil mass per volume) per peatland for both current rooting zone soil (active layer, AL) and near-surface permafrost soil (PF).

Peatland	Depth	Bulk density *(mgDW/cm^3)*	
		Mean	*SE*
A. **Stordalen**	AL	57.4	4.2
	PF	134.8	18.5
B. **Torneträsk**	AL	57.6	9.3
	PF	205.8	5.1
C. **Storflaket**	AL	84.4	11.3
	PF	209.7	43.9
D. **Abisko**	AL	36.8	2.4
	PF	221.8	28.7
E. **Kursflaket**	AL	38.2	2.2
	PF	275.3	121.4
All peatlands	AL	274.4	4.5
	PF	1047.3	19.4

The values in the main text of this article are expressed as mg N per m^2 per 10 cm depth, which reflects the amount of plant-available nitrogen that may be released yearly in the near-future (e.g. decade, based on an annual 1 cm thaw rate (Akerman & Johansson 2008)). This is equivalent to 0.1 mg N m^{-3} and can be recalculated into other units for comparison with literature with the bulk density data provided in Table SI 1.

Total N values were calculated by multiplying the %N by weight with the bulk density. Note: total N represents all N, including non-extractable highly recalcitrant N-forms, whereas DON, DIN and N_{mic} (Table 2 in the main document) are all extractable N forms only. Hence, total dissolvable N is lower than total N.

Mean total N values per 10 cm soil depth were 41 g m^{-2} (± 6 SE) for current rooting zone soil and 297 g m^{-2} (± 20 SE) for near-surface permafrost soil. Total N was analysed with repeated-measures ANOVAs for the effects of depth (within-subject factor: current rooting zone, permafrost) and sampling location (between-subject factor, five peatlands): factor depth F = 259.24, $P < 0.001$; peatland $F = 6.64$, $P < 0.01$ and a significant interaction between the two main factors depth and peatland $F = 3.97$, $P < 0.05$.

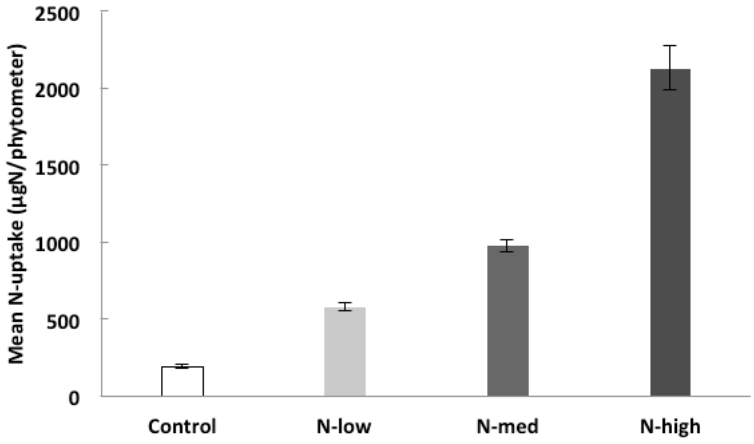

Figure SI 1. As a positive control of *Poa alpina* phytometer N-limitation, three N additions of 0.75 g N m^{-2} (N-low), 1.5 g N m^{-2} (N-med) and 3.0 g N m^{-2} (N-high) were applied. Fertilizer was given to the soil surface (79 cm^2) as NH_4NO_3 solution divided over 6 weekly 10 ml gifts. Controls, soil and litter treatments received 10 ml de-ionized water simultaneously to prevent moisture-related artefacts. Data are means ± SE, $n = 13$, all treatments differed significantly from each other ($P < 0.001$).

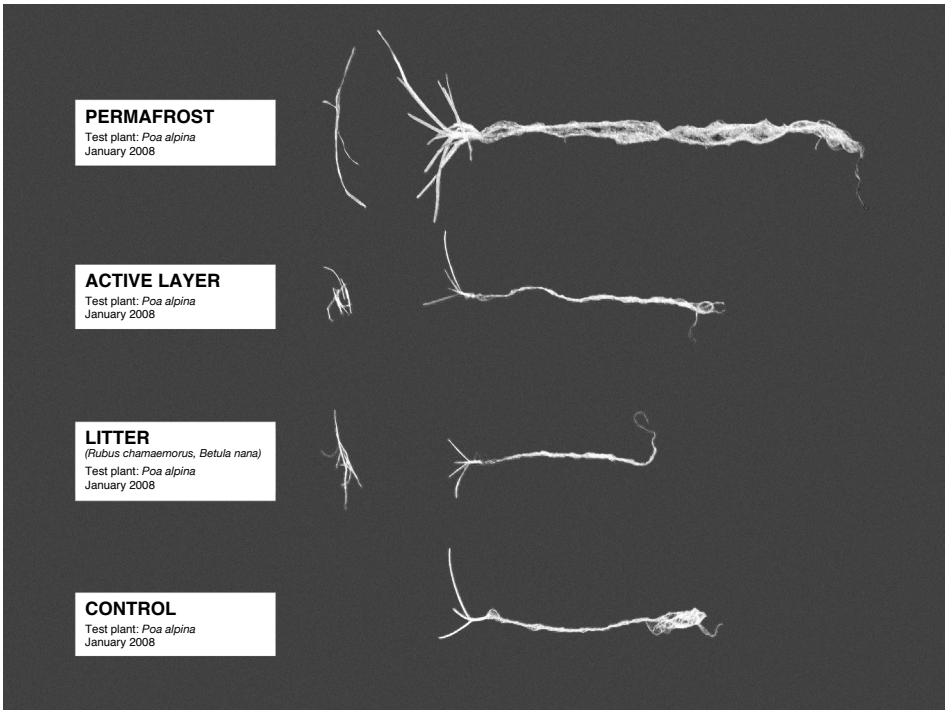

Figure SI 2. Phytometers grown on sand mixed with near-surface permafrost soil, current rooting zone (active layer) soil or litter, immediately after their harvest, showing remarkable differences in size. For an elaborate description of the historical use, pros and cons of the bioassay technique see Axmanova *et al.* (2011, *Plant and Soil*, 342, 183-194).

Table SI 2. Averages and *F*- statistics for repeated-measures ANOVAs for the effects of depth and sampling location on potential enzyme activities for four aminopeptidases in peat soils (see text for abbreviations) incubated at 4 °C (nmol/gDW/hr). Sampling location (peatland A-E) was taken as between-subject factor and depth (active layer, permafrost) as within-subject factor. Alanine data were log-transformed before analysis.

Substrate		Mean	SE	Depth	Peatland	Depth X Peatland
Alanine	*AL*	7.85	1.29	3.74+	0.79	3.02*
	PF	15.47	3.91			
Lys.ala	*AL*	3.35	0.25	21.02***	2.44	5.39**
	PF	6.58	0.59			
Leucine	*AL*	6.03	0.55	12.78**	2.51+	3.18*
	PF	11.16	1.18			
AAP	*AL*	318.08	40.50	36.53***	1.28	1.27
	PF	63.82	10.20			

Methods fluorometric enzyme assays

Enzyme assays were conducted according to the protocol of Steinweg and McMahon (http://enzymes.nrel.colostate.edu/) using substrates labelled with 7-amino-4-methylcoumarin (MUC). Specific substrates were L-leucine-7-amido-4-MUC (Leucine), L-alanine-7-amido-4-MUC (Alanine), L-lysine-alanine-7-amido-4-MUC (Lys-Ala), and L-alanine-alanine-phenylalanine-7-amido-4-MUC (AAP) (all substrates supplied by Sigma-Aldrich).

Soil slurries were prepared by blending 4 g (fresh weight) homogenized peat in 90 mL of 50 mM sodium acetate buffer (pH 5) for 60 seconds. 800 μL of slurry were incubated with 200 μL of 200 μM substrate in 96-deepwell plates at at 4 °C for 20 hours. Simultaneously 800 μL slurry was incubated in identical conditions with 200 μL volumes of a dilution series of MUC standard (0, 2.5, 5, 10, 25, 50, 100 μM). In this way standard curves could be constructed for each sample, thus controlling for between-sample variance in fluorescence quenching dynamics. After incubation, plates were centrifuged at 1500 rpm for 3 minutes, the supernatant transferred to fluorescence plates and pH adjusted with 5 μL 0.5M NaOH. Fluorescence was then immediately measured using a Spectramax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, USA) with excitation wavelength 365 nm and emission wavelength 450 nm. Product accumulation was calculated by comparison with each sample's standard curve. When standard curves were non-linear separate curves were fitted to the separate linear portions and the appropriate regression equation used for calculating sample reaction rates. All measurements were converted to nanomols per gram dry weight per hour.

IV